Keeping an eye on coloration: ecological correlates of the evolution of pitcher traits in the genus Nepenthes (Caryophyllales)

Title: Keeping an eye on coloration: ecological correlates of the evolution of pitcher traits in the genus *Nepenthes* (Caryophyllales)

Running title: Pitcher evolution in *Nepenthes*

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Abstract
Nepenthes is a genus of carnivorous pitcher plants with high intra- and interspecific morphological diversity. Many species produce dimorphic pitchers, and the relative production rate of the two morphs varies interspecifically. Despite their likely ecological importance to the plants, little is known about the selective context under which various pitcher traits have evolved. This is especially true of color-related traits, which have not been examined in a phylogenetic context. Using field observations of one polymorphic species (*N. gracilis*) and phylogenetic comparative analysis of 85 species across the genus, we investigate correlations between color polymorphism and ecological factors including altitude, light environment, and herbivory. In *N. gracilis*, color does not correlate to amount of prey-capture, but red pitchers experience less herbivory. Throughout the genus, color polymorphism with redder lower pitchers appears to be evolutionarily favored. We found a lack of phylogenetic signal for most traits, either suggesting that most traits are labile or reflecting the uncertainty regarding the underlying tree topology. This work highlights ecological correlates of the vast phenotypic diversity of this group of tropical plants. We point to a need for future work examining herbivores of *Nepenthes* and experimental investigations on color polymorphism.

Key Words: altitude - carnivorous plants - coloration - comparative methods - herbivory - intraspecific diversity - *Nepenthes* L. - pitcher plants - plant-animal interactions

Introduction:

Competition for resources can lead to the divergence of a clade into multiple niches and the evolution of novel morphological features. This can be seen in many plant radiations such as the bromeliads, where the species that came to occupy water- and nutrient-limited habitats evolved tightly pressed leaf tanks capable of collecting water and nutrient-rich debris (Benzing & Renfrow, 1974; Givnish et al., 2011). In addition to resource limitation and/or competition, plants must also routinely respond to a suite of interspecific interactions. For instance, animal pollinators have a prominent role in shaping floral evolution (e.g. Muchhala & Potts, 2007; Kay & Sargent, 2009; Alcantara & Lohmann, 2011; van der Niet & Johnson, 2012; Anderson et al., 2014; Boberg et al., 2014; Muchhala, Johnsen, & Smith, 2014; Lagomarsino et al., 2016). Thus plant morphological evolution can have multiple, interacting biotic and abiotic drivers. However, disentangling the effects of these various drivers is difficult and has not been achieved in many groups.

Pitcher plants are one such group characterized by an adaptation that is subject to multiple interacting drivers. Their pitchers are modified leaves used to capture and digest animal prey—they are nitrogen-acquiring organs analogous to bromeliad tanks, but are also like flowers in their potential to coevolve with animal visitors. Pitcher plants are thus a useful system to investigate the roles of abiotic and biotic effects on the diversification of an adaptive trait. Here we examine the carnivorous plant genus *Nepenthes* L., the most diverse (>140 species: Cheek & Jebb, 2014) and widespread family of carnivorous pitcher plants. Its core distribution spans most of Southeast Asia, with a few outlying species in Madagascar to the west and New Caledonia to the east, and ranges in altitude from 0 up to 3520 m asl (McPherson, Robinson, & Fleischmann, 2009). The pitchers of different species are used to prey on insects, but additionally may be

involved in interactions spanning from commensalism to parasitism and mutualism (e.g. Beaver, 1979; Adlassnig, Peroutka, & Lendl, 2011; Bazile et al., 2012; Thornham et al., 2012; Scharmann et al., 2013). Pitchers are morphologically complex, exhibiting an array of traits that are under selection by both biotic and abiotic factors that are difficult to tease apart.

Recent work has elucidated the functional significance of pitcher traits including the thickness of the peristome, presence of a waxy layer, viscosity of the fluid, and digestive gland structure in relation to prey trapping efficiency (Bonhomme et al., 2011; Renner & Specht, 2011; Bauer et al., 2012). Although it has not yet been well documented whether interspecific differences can be explained by niche-partitioning (Chin, Chung, & Clarke, 2014; but see Peng & Clarke, 2015; Gaume et al., 2016), the few known specialist trappers point to the importance of animal visitors to pitcher phenotypic evolution; this includes traits such as the parabolic structure in *N. hemsleyana* Macfarl. that functions as an echolocation guide for its mutualist bat (Schöner et al., 2015; Schöner et al., 2017). Despite growing knowledge of the significance of different trapping features to the genus, less than 10% of all species have been the subject of ecological studies detailing their specific prey capture strategies (Clarke and Moran, 2011), and the functional significance of many pitcher traits has yet to be explored.

One potentially important, yet understudied set of traits in pitcher plants are those related to intraspecific polymorphism. Many species produce two distinct pitcher morphologies ("morphs") throughout the lifespan of an individual plant: lower pitchers ("lowers"), which grow gravitropically from plants in the rosette phase and possess winged fringes of tissue ("wings"); and upper pitchers ("uppers"), which grow from plants in the climbing phase, twine onto surrounding vegetation via their tendrils, and possess a more streamlined form lacking wings (Jebb and Cheek, 1997; Figure 1). There is some evidence of prey-partitioning between pitcher

morphs (Moran, 1996; Rembold et al., 2010; Gaume et al., 2016) and differences in symbiont communities of different pitcher morphs (Clarke, 1997a), but little is known about the evolution or functional significance of pitcher dimorphism.

In addition to shape, many species also have pitchers that vary from red to green, often with discrete color differences between the lower and upper morphs (Figure 1—we hereafter refer to the occurrence of discrete color differences between pitcher morphs within a plant as "color polymorphism"). Despite being a conspicuous feature of *Nepenthes*, pitcher coloration is poorly understood. A few studies have examined the role of red pigmentation as a visual signal in carnivorous plants (Schaefer & Ruxton, 2008; Bennett & Ellison, 2009; Foot, Rice, & Millet, 2014; Jürgens et al., 2015; El-Sayed, Byers, & Suckling, 2016), and a number have hypothesized that the contrast of red against a green background of foliage could be attractive, although many insects lack red perception (Briscoe and Chittka, 2001). Red was not found to be a prey attractant in studies with sundews (Foot, Rice, & Millet, 2014; Jürgens et al., 2015; El-Sayed, Byers, & Suckling, 2016), but results have been conflicting in pitcher plants (Schaefer & Ruxton, 2008; Bennett & Ellison, 2009).

As red pigmentation in *Nepenthes* is due to anthocyanins (Kováčik, Klejdus, & Repčáková, 2012), which are costly to produce (Gould, 2004), the existence of intraspecific color polymorphism in pitcher plants is particularly puzzling. More generally speaking, the role of plant anthocyanins as visual signals in flowers and fruits is well understood, but the function of anthocyanins in leaves is less resolved. Multiple competing, though not necessarily mutually exclusive, hypotheses have been proposed for the role of anthocyanins in leaves (Gould, 2004), the majority of which can be divided into two camps: those that argue that anthocyanins primarily serve a physiological role, vs. those that posit that they are visual signals and primarily

a result of coevolving with herbivores (Archetti & Brown, 2004; Archetti et al., 2009). Some potential physiological functions of anthocyanins involve protecting leaves from excess light, including UV shielding and free radical scavenging (Feild, Lee, & Holbrook, 2001; Hoch, Zeldin, & McCown, 2001; Neill & Gould, 2003), and facilitating nutrient resorption in the context of deciduous color-changing leaves (Hughes, Singsaus, & McCown, 2003). Additionally, abaxial anthocyanins in understory plants have been proposed to improve photosynthetic efficiency in the "back-scatter hypothesis", though there is some experimental evidence against this (Hughes, Vogelman, & Smith, 2008). Considering the coevolution hypothesis, anthocyanins may serve as either direct (Schaefer, Rentzsch, & Breuer, 2008; Tellez, Rojas, & Van Bael, 2016) or indirect (Page & Towers, 2002; Archetti & Brown, 2004; Karageorgou & Manetas, 2006; Schaefer & Rolshausen, 2006; Lev-Yadun & Gould, 2008; Archetti et al., 2009) defense against herbivores and pathogens.

Here, we seek to better understand the functional significance and diversification of dimorphic traits in pitchers using two complementary approaches: (1) a field study of the polymorphic species *N. gracilis* Korth., exploring the functional significance of intraspecific variation in pitcher traits and (2) a comparative phylogenetic analysis of species across the genus exploring trait evolution more broadly. This approach should allow us to identify broad patterns across the genus that can be verified in more detail within a particular species.

Our study of *N. gracilis* tests the following hypotheses: 1) red pigmentation promotes prey capture and/or symbiont colonization; 2) red pigmentation increases with increasing light intensity; 3) red-pigmented pitchers show fewer signs of herbivory. Our comparative phylogenetic study first tests for phylogenetic signal in pitcher traits (Felsenstein, 1985). We then use stochastic character mapping to determine if particular color states are evolutionarily

favored. Finally, we test for the correlation of pitcher traits with each other and with environmental traits, including habitat type and altitude. Moran et al. (2013) found precipitation to be a key factor behind the distribution of the traits they examined (peristome width, wax presence, and presence of viscoelastic fluid). Furthermore, previous studies hypothesize that the decreasing availability of ants with altitude (Hölldobler & Wilson, 1990) increases selective pressure for evolving specialized dietary strategies (Clarke et al., 2009), which could impact many pitcher traits including coloration and dimorphism. As both precipitation and ant abundance covary with altitude, so we analyze the role of altitude as a primary abiotic driver of trait evolution. In addition to altitude, we explore habitat and growth habit as proxies for abiotic drivers of coloration evolution.

Methods:

1.**Intraspecific variation in *N. gracilis***

a. **Field sites**

Singapore (1.5° N) is aseasonal, with an average annual rainfall of 2,340 mm, an average minimum diurnal temperature of 25° C, an average maximum diurnal temperature of 37° C, and relative humidity levels generally above 90% in the morning and down to 60% later in the day. The highest point in Singapore is 165 m asl (Bukit Timah Hill). The natural areas utilized in this study include Kent Ridge Park (1°17'13.00"N, 103°47'10.91"E) and MacRitchie Reservoir Park (1°20'34.99"N, 103°49'47.96"E). Kent Ridge Park is a secondary “adinandra belukar” forest, dominated by simpoh air (*Dillenia suffruticosa* Martelli) trees. Adinandra belukar type vegetation is characterized by acidic soils (3.3 – 3.9 pH) and low nitrogen and phosphorous (Chan et al., 1997). The MacRitchie Reservoir Park pitcher plants examined grow on the coast of

an artificial water reservoir supported by *Ploiarium alternifolium* Melch. and simpoh air trees. *Nepenthes gracilis* is abundant throughout natural areas in Singapore. We chose this species for its abundance and high level of intraspecific variability. We specifically worked in microhabitats where *N. gracilis* grew isolated from its local congeners (*N. ampullaria* Jack and *N. rafflesiana* Jack).

b. **Assessment of insect accumulation rates in different pitcher variants**

In mid-July 2014, pitchers were sampled from two separate areas within Kent Ridge Park separated by about 0.3 km and at one site in MacRitchie Reservoir, which is about 8 km from Kent Ridge. As we could not know how long each pitcher had been open prior to our survey, we needed to "reset" all of the pitchers in our study sites to be able to compare arthropod colonization rates across pitchers given equal time. We first emptied each pitcher, marked it with a small tag attached to the base of the lamina distal from the pitcher, and then returned to collect its entire fluid contents one month after emptying. Arthropods contained in the pitchers were filtered out from the fluid and stored in 100% ethanol prior to being counted and identified according to higher level classification (e.g. order or family depending on the taxon) under a dissecting scope.taxa under a dissecting scope. We recorded the following characteristics from each sampled pitcher: pitcher morph (upper or lower), pitcher color (red or green), pitcher condition (healthy or damaged/senescent), the length and width of the pitcher, fluid volume, its distance from the ground, a rank of "connectedness" (degree to which pitchers formed physical connections with surrounding plants via twining, scored from 1-3, with 1 being no connection to other vegetation and 3 being fully twined and well-connected), and the node on which the pitcher occurred.

To determine whether counts of insect prey and symbionts differed significantly between pitchers of differing traits, we performed Poisson regressions using the 'glmer' function in the 'lme4' package (Bates et al., 2014) in R 3.3.2 (RCoreTeam, 2013). We collected from multiple pitchers per plant, so we set plant as a random effect, as well as collection site. We included all examined traits (pitcher color, pitcher morph, connectedness, pitcher size, and distance from the ground) as fixed effects in one model in order to account for any correlations among traits. To avoid the potential confounding effects of senescence or increased herbivory, pitchers that deteriorated in condition over the one month period after emptying were excluded from the analysis We tested for statistically significant differences in numbers of ants, culicid larvae (mosquitoes), non-culicid larvae (all low-abundance dipteran taxa), mites, and flying prey items between pitchers that differed in all of the aforementioned measured pitcher characteristics.

c. **Assessment of relationship between pitcher color and canopy coverage**

In January 2014, for 8 arbitrarily-selected locations within Kent Ridge Park, we laid out plots of approximately 1.5 m in diameter and then exhaustively tallied all of the pitchers within the plots. Based on morph and color, pitchers were assigned to one of four categories: red lower, red upper, green lower, and green upper. We estimated the canopy coverage by photographing the canopy above each plot (pointing upwards from the level of the pitchers at the center of the plot) using a digital camera (Canon PowerShot ELPH 170IS) and calculating the total area of shade-free space in each image by counting white cells using 625 $pixel^2$ per cell grids in ImageJ (Rasband, 2012). We tested for a correlation between canopy coverage and the proportion of red pitchers per plot using a linear regression.

d. **Assessment of relationship between pitcher color and herbivory**

In late January to early February 2016, we tallied pitchers within 8 plots in Kent Ridge Park as described above. To test for a relationship between red pigmentation and herbivory in *N. gracilis*, we scored each pitcher within a plot for pitcher type (the four categories of color and morph described above) and for whether or not the pitcher exhibited signs of herbivory or pathogen attack. Pitchers were scored as having signs of attack based on the presence of localized spots of discolored, senescent, or missing tissue anywhere on the pitcher body (this was treated as a binary character, so any pitchers lacking such signs were scored as "not attacked"). We performed a logistic regression using the 'glmer' function in the 'lme4' package (Bates et al., 2014) in R 3.3.2 (RCoreTeam, 2013) to test for a relationship between pitcher color and signs of attack, including plot as a random effect. We also performed a logistic regression in the same way on the subset of lower pitchers to examine the effect of color while controlling for morph. To test for a relationship between pitcher morph and signs of attack while controlling for color, we performed a logistic regression on the subset of green pitchers. The number of red upper pitchers (n=1) was too small to meaningfully compare red uppers and lowers or red and green uppers.

2. Comparative analysis of interspecific variation in Nepenthes

a. Sequence mining and phylogenetic inference

Previous molecular studies of the genus have utilized different markers: the peptide transferase single copy nuclear gene (*PTR1*: Meimberg & Heubl, 2006), the plastid *trnK* intron (Meimberg et al., 2001, 2006; Merckx et al., 2015) and the nuclear ribosomal transcribed spacers (*nrITS1*-

5.8S-nrITS2, (Alamsyah & Ito, 2013; Schwallier et al., 2016). Only those studies using *PTR1* and *trnK* shared voucher specimens, so these were the two markers we chose for phylogenetic inference to ensure the taxonomic identity of the specimens was consistent between sequences, especially considering that the risk of misidentified sequences is a caveat inherent to the use of sequences obtained from a database. While currently available sequence data have proven insufficient to conclusively resolve the phylogeny of *Nepenthes* (Meimberg et al., 2001, 2006; Alamsyah & Ito, 2013), they nevertheless provide a working hypothesis with which to begin looking for patterns. Sequences were downloaded from Genbank, resulting in 87 sequences for the ~2500 bp *trnK* plastid gene region, and 40 sequences for the ~1605 bp *PTR1* nuclear gene. We did not use sequences for the pseudogenized copy of the *trnK* gene (Meimberg et al., 2006). Our outgroups were *Triphyophyllum peltatum* and *Ancistrocladus abbreviatus*, which both have *trnK* sequences (Meimberg et al., 2001). Specimen information and sequences used are summarized in Supplemental Table 1. Sequences for each of the genes were aligned separately using MUSCLE (Edgar, 2004) in the Geneious 7.0 platform. To remove ambiguously aligned regions, Gblocks 091 with relaxed parameters (Castresana, 2000; Talavera & Castresana, 2007) was applied to the *trnK* alignment. Best-fitting models for DNA substitution for each marker were selected according to the corrected Akaike information criterion (AICc) in jModeltest ver. 0.118 (Posada, 2008). These resulted in GTR+G for *trnK* and GTR+I for *PTR1*.

An ultrametric tree was inferred using Bayesian MCMC in the program BEAST v1.8.3 (Drummond et al., 2012). A Yule tree prior model and a strict clock were applied (as no definitive fossils of Nepenthaceae are known, no fossil calibration points were used), and two independent chains were run for 10 million generations. Convergence was inspected in Tracer v.1.5 and a 10% burn-in was applied to each chain to obtain the final tree.

b. Character matrix

A character matrix was gathered from species descriptions in McPherson, Robinson, & Fleischmann (2009), which includes accounts of 125 species and incompletely diagnosed taxa. Using a single source has the advantage of greater consistency in the scoring of characters, in particular those related to color. Scoring of such traits may be subjective and vary between accounts; furthermore, original species descriptions do not always describe color variation in depth or provide color photographs. Another problem with this source is that the information on color variation within species is based on qualitative descriptions as opposed to quantitative descriptions of the proportions of color variants within pitcher morphs. Some species have variable coloration, and without data on the proportions of color variants, both morphs may be described as "variable", which may mask finer details (i.e. whether the two morphs have different probabilities of being red); however, this still allows us to examine broad patterns. We note that this field guide is not a peer-reviewed source, so wherever possible we have also cross-checked this information against the Jebb and Cheek (1997) *Nepenthes* monograph. We have also included some additional data (peristome width/slope and viscosity) from Bauer et al. (2012) for further comparison. Finally, our data are constrained to colors that are found in the visible spectrum. Certain pitcher plant species are known to be strongly reflective and/ or absorbing in the UV as well as long wavelength (e.g. Joel et al., 1985; Moran et al., 1999), and the UV in particular may be important in signals involving insects. However, since only a few pitcher plant species have been assessed for their spectral qualities outside the visible, we were unable to include a wider range of wavelengths in our analysis.

In our scoring for color polymorphism, we scored species either as “redder lower”, “redder upper”, or “similar coloration”. All of these scores deal specifically with levels of red pigmentation. If a species produces mostly solid-colored pitchers and the lower pitchers are generally red (to the human eye) and the upper pitchers are generally green (to the human eye), then it was scored as “redder lower”—the reverse was scored as “redder upper”. For species with patterning (blotches, spots, stripes, or mottles of red/dark pigmentation on the outer pitcher wall), the pitcher morph with denser pigment patterning was considered to be “redder”. Darker colored pigmentation was assumed to be the result of increased expression of anthocyanin, so a morph with solid or patterned “black”, “purple”, or “brown” color was considered to be redder than a morph with solid or patterned “red”, “orange”, or “pink” color. In cases of variation within a pitcher morph, the most commonly observed coloration was used for the comparison. Species with pitcher morphs that are deemed to be generally equivalent for all of the above-described properties were scored as “similar coloration”. Species where both pitcher morphs exhibit color variation and both pitcher morphs are described as “equally variable” were also scored as ‘1’ for “similar coloration”.

“Lid contrast” and “peristome contrast” refer to whether the lid/peristome differs in coloration from the pitcher body; e.g., a green pitcher body with a red lid/peristome or a red pitcher body with a green lid/peristome. The underside of the pitcher lid is generally lighter in color than the outer wall of the pitcher body, so this did not factor into the scoring of this set of characters. However in terms of increased pigment relative to the body, a pitcher was scored as having “lid contrast” based on either the entire lid, the upper surface of the lid, or the under surface of the lid—wherever the strongly contrasting red or green coloration is expressed. The contrast scores for lids/peristomes were based primarily on solid colors and any spots or stripes

were not considered. Peristome striping was scored as a binary trait, where the trait was scored as present whenever any expression of the trait is reported in a given species. All patterning traits were scored independently for each pitcher morph.

We scored presence/absence of pitcher dimorphism and a related yet distinct trait we refer to as "reduced lower pitcher production" or "reduced lowers". These species still produce both morphs, except that they only produce lowers in young plants and then switch to solely producing uppers, as opposed to other species that continue to produce both when mature. Both pitcher dimorphism and reduced lowers were scored as binary.

We scored each of three growth habits (terrestrial, epiphytic, lithophytic) and each of nine habitats (lowland dipterocarp forest, peat swamp, heath forest, montane forest, scrub, cliff, mangrove, seasonal grassland, and degraded—which includes all anthropogenically-modified environments) as binary traits, denoting the presence or absence of a species in that habit/habitat.

Species designations follow the taxonomy of McPherson, Robinson, & Fleischmann (2009). Given this, the Meimberg et al. (2001) accession named as *N. anamensis* was scored according to the McPherson, Robinson, & Fleischmann (2009) account of *N. smilesii*. *Nepenthes xiphioides* and *N. pectinata* were both scored identically to *N. gymnamphora*. As what Meimberg et al. (2001) designates as *N. pilosa* is likely *N. chaniana* (Clarke, Lee, and McPherson, 2006), we have relabeled their "*N. pilosa*" sequence as "*N. chaniana* 3" ("*N. chaniana*" and "*N. chaniana* 2" are from Merckx et al., 2015 data).

c. Phylogenetic tests

All statistical analyses were conducted in R 3.3.2 (R Core Team 2013). We tested for phylogenetic signal in continuous traits with Blomberg's K (Blomberg, Garland, & Ives, 2003) and Pagel's lambda (Pagel, 1999) using the 'phylosig' function in the 'phytools' package

(Revell, 2012), and in binary traits with Fritz and Purvis's D (Fritz & Purvis, 2010) using the 'phylo.d' function in the 'caper' package in R (Orme, 2013). To find the number of transitions between states for color polymorphism we used the 'countSimmap' function using the 'phytools' package in R; this method is a form of stochastic character mapping and has the advantage of accounting for uncertainty in the underlying topology (Bollback, 2006). To test for the influence of altitude on pitcher traits, we performed a phylogenetic generalized least squares (pgls; for continuous traits) using the 'pgls' function in the 'caper' package and utilized the 'brunch' function (for discrete traits) in the 'caper' package in R. We used pgls to examine correlations between morphological traits. To test for correlated evolution between color polymorphism and patterned pitchers, between reduced lowers and color traits, and between various traits and habitat/growth habit, we used a binary PGLMM (phylogenetic generalized linear mixed model) using the ape package (Paradis et al. 2016).

Results:

Intraspecific variation in *Nepenthes gracilis*

At Kent Ridge Park and our site in MacRitchie Reservoir, we collected all the fluid and associated organisms from 83 pitchers of *Nepenthes gracilis* (31 individual plants, Supplemental Table 2). We counted total of 822 pitchers in Kent Ridge Park during our January 2014 survey of *N. gracilis* in relation to canopy coverage, and a total of 605 pitchers during our January 2016 survey of *N. gracilis* in relation to herbivory.

Our data show no significant differences in counts of prey (ants, mites, and flying prey) or symbionts (culicids and other larvae) explained by pitcher color, morph, connectedness, distance

from the ground, or size (Poisson regression, $p>0.05$ in all cases, Supplemental Table 3), except that pitcher size is positively correlated with number of ants ($p<0.05$, Supplemental Table 3).

From our January 2014 survey, we found that lower pitchers were disproportionately more likely to be red-pigmented than upper pitchers (chi-squared test, $chi^2= 148.3$, $p<0.001$; Supplemental Table 4), showing that *N. gracilis* has "redder lower" color polymorphism. We also found a significant positive correlation between the proportion of red pitchers in a site and canopy cover ($r^2=0.79$, $p<0.01$, Figure 2). From our January 2016 survey, we found that red pitchers were disproportionately less likely to show signs of herbivore or pathogen attack in the field (logistic regression, $p=0.002$). This result was similar when accounting for pitcher morph by only comparing red and green lowers (logistic regression, $p=0.002$). However there was no difference in the likelihood of attack due to pitcher morph, either in the full dataset (logistic regression, $p=0.72$) or between the green subset of uppers and lowers (logistic regression, $p=0.47$).

Phylogenetic inference

The phylogeny we constructed using the *trnK* and *PTR1* genes (Figures 3-4) is similar to the phylogenies published by Meimberg et al. (2001) and Meimberg and Heubl (2006). The first split within *Nepenthes* separates a clade consisting of *Nepenthes khasiana* and *N. madagascariensis* + *N. masoalensis* from the remaining species, which are then split into a clade consisting of *N. pervillei* + *N. distillatoria* and the rest of *Nepenthes*. These two smaller clades include the "outlying" species from the Western limits of the genus' range (India, Madagascar, the Seychelles, and Sri Lanka), which have appeared in a similar position in all of the

phylogenies published thus far (often referred to as "basal" species in previous studies, e.g. Meimberg et al. 2001, 2006; Alamsyah & Ito, 2013). The branch lengths become much shorter and less well-resolved for the numerous species from the Southeast Asian center of distribution. Within this large Southeast Asian clade, a clade consisting mostly of Papuan species is sister to the remaining species. As with previous studies, however, several nodes are poorly resolved, particularly within the aforementioned large Southeast Asian clade containing species from Sundaland, the Philippines, and western Wallacea.

Phylogenetic signal

None of the quantitative traits we examined exhibit significant phylogenetic signal, neither with Pagel's lambda nor with Blomberg's K (Table 1). None of the binary traits we examined exhibit significant phylogenetic signal (Table 2), except for lower peristome stripes (probability random distribution= 0.003). The lack of significant signal in the majority of these traits may suggest evolutionary lability in *Nepenthes* pitcher evolution or may equally plausibly be attributed to the lack of topological resolution inferred from the currently available genetic data.

State switches in color polymorphism

Our analysis of state switches in the color polymorphism trait yielded "redder lowers" as the state with the longest evolutionary residence time, followed by "similar coloration", and the shortest time for "redder uppers" (proportion of time spent in state 0.52, 0.31, and 0.17. respectively). Switches happening between "redder lowers" and "similar coloration" are more

numerous than any of the switches involving “redder uppers”. Switches away from “redder uppers” are more numerous than switches to “redder uppers”. Switches between “redder lowers” and “redder uppers” are more numerous than those between “similar coloration” and “redder uppers”. Overall, together with the likelihood that “redder lowers” is ancestral, these trends imply that “redder lowers” is the default state and “redder uppers” is evolutionarily disfavored relative to the other two states. (Figure 5)

Correlations with altitude and between morphological traits

None of the quantitative or binary traits we examined exhibit a significant relationship with altitude (pgls, Table 3). Lamina length increases with lower pitcher length (pgls, $p<0.001$, Table 3) and lower peristome rib height increases with upper peristome rib height (pgls, $p<0.0001$, Table 3).

Tests of correlated evolution: color polymorphism, reduced lowers, and habit/habitat

We found no significant correlated evolution between color polymorphism and pitcher dimorphism, reduced lower pitchers, or patterned pitchers (binary PGLMM, $p>0.05$ in all cases; Supplemental Table 5). The “reduced lowers” trait is positively correlated with the “similar coloration” trait (binary PGLMM, correlation estimate =1.70, $p = 0.003$; Supplemental Table 6) and negatively correlated with the “redder lowers” trait (binary PGLMM, correlation estimate = -1.72, $p = 0.003$; Supplemental Table 6). Color-related traits in general show no significant correlations with growth habit or habitat (binary PGLMM, $p>0.05$ in all cases; Supplemental

Tables 7). There is a significant positive correlation between reduced lowers and epiphytism (binary PGLMM, correlation estimate = 5.90, p = 0.002; Supplemental Tables 7).

Discussion:

Species of *Nepenthes* represent a plant radiation with high morphological and ecological diversity in their pitchers. We took two approaches to evaluating the ecological drivers of dimorphism- and color-related morphological traits in *Nepenthes*: a field study of the polymorphic species *N. gracilis* and comparative phylogenetic analysis across the genus. Our field studies of *N gracilis* showed the potential importance of light environment and herbivore pressure to color polymorphism, with lower pitchers disproportionately more likely to be red-pigmented than upper pitchers (Supplemental Table 4), and the proportion of red pitchers in a site significantly correlated with canopy cover (Figure 2). Our comparative analysis further showed that redder lower pitchers may be evolutionarily favored across the genus. We found little evidence supporting that altitude, growth habit, or habitat are key drivers of the traits we examined (Supplemental Tables 7). We further discuss our results below.

Pitcher dimorphism

We found no evidence that dimorphism is correlated to altitude (Supplemental Tables 7), contrary to our expectation that dimorphism would be lost with the decreasing availability of ants at higher altitudes. We also examined situations in which the production of lower pitchers is reduced. We expected this trait to increase with altitude, but we again found no significant relationship (Supplemental Tables 7). However, we found that the evolution of reduced lower pitchers is positively correlated with the evolution of epiphytism (Supplemental Tables 7),

possibly reflecting that upper pitchers are better suited to an arboreal environment than lowers. Interestingly, only one of the species in our dataset is a strict epiphyte—the rest grow terrestrially as well—so reduced lowers may be a means of entering an epiphytic niche rather than a consequence of becoming epiphytic.

We found no difference in rate of prey capture between upper and lower pitchers of *N. gracilis* in our observations of this species, and no evidence for partitioning of crawling and flying insects between morphs (Supplemental Table 3). This is consistent with the results of Gaume et al. (2016), a study examining prey capture in seven sympatric Bornean taxa with morphological differences, where *N. gracilis* showed far less difference in prey composition of upper and lower pitchers than the other species examined. This shows that while dimorphism may have a pronounced ecological role in some species, this pattern is not universal throughout the genus.

Color polymorphism

We tested three hypotheses for the function of red pitchers in *N. gracilis*: (1) red coloration acts as a visual signal to prey and symbionts, (2) red pigmentation protects pitchers from excess solar radiation, and (3) red pigmentation is related to defense.

We found no support for our first two hypotheses: red and green pitchers did not differ in their prey capture rates in *N. gracilis* (Supplemental Table 3), and red pitchers were significantly less common in areas with greater sun exposure (Figure 2). The lack of difference in prey capture between red and green pitchers makes sense from the perspective of insect vision: ants, the main prey items, lack an ability to perceive red (Briscoe and Chittka, 2001). Our finding on sun exposure is the opposite from what we would predict if the anthocyanins in red pitchers

function primarily to protect against UV. This does not necessarily rule out all possible physiological functions (e.g., protection against sun flecks; Gould et al., 1995), but pitchers are also less photosynthetically active than the laminae (Pavlovič, Masarovičová, & Hudák, 2007; Pavlovič et al., 2009; Adamec, 2010a,b), further diminishing the likelihood of a photosynthetic function. However, our observations were consistent with our third hypothesis, that red pigmentation is related in some way to defense.

Defense is likely prioritized more as plants become more nutrient-stressed or energy-limited (Gianoli, 2015). In Moran & Moran (1998), it was shown that red coloration in *N. rafflesiana* can be induced by nutrient stress. In our study, red pitchers were more likely to occur in the shade, and showed significantly fewer signs of herbivory than green pitchers (logistic regression, $p<0.01$). Thus pigmentation could play a defensive role in *N. gracilis,* possibly an indication that red pitchers are more chemically defended (Menzies et al., 2016), or less nutritionally valuable, and/or that the coloration defends against herbivores via crypsis (Fadzly et al., 2009; Klooster, Clark, & Culley, 2009; Fadzly & Burns, 2010; Niu et al., 2014; Fadzly et al., 2016), which is plausible considering that lowers often grow in reddish-brown leaf litter while uppers tend to grow embedded in green foliage (K. Gilbert, pers. obs.). The greater likelihood for lowers to be red compared to uppers also supports the defense hypothesis, as a climbing habit reduces herbivore pressure (Gianoli, 2015). The results of our phylogenetic analyses suggest that the selection for redder lowers we see in *N. gracilis* may be generalizable to the genus as a whole. Not only is the number of clades with redder lowers much greater than those with redder uppers, but the "redder uppers" state has the lowest residence time in our state switch analysis (Figure 5), suggesting that pigmented upper pitchers are generally not evolutionarily favored.

Another line of evidence for the putative role of herbivory in shaping color polymorphism is that the evolution of reduced lowers is associated with the loss of color polymorphism and a shift away from "redder lowers" (Supplemental Table 6). A lessened investment in producing lowers could conceivably lead to a lessened investment in pigmenting them. Alternatively, as species with reduced lowers have a tendency towards epiphytism, both morphs may experience more similar environmental conditions than usual, leading to similar coloration. It is notable that epiphytes completely avoid the relatively higher herbivore pressure of terrestrial areas (Gianoli, 2015), so the defensive role of pigmentation would be relaxed. When both pitcher morphs are red in epiphytes, it could be due to a stronger signaling role (see *N. macrophylla* in Moran et al., 2012).

The evolution of contrasting color patterns within pitchers

In addition to examining color polymorphism, we explored the evolution of interspecific diversity in contrasting color patterns, which include a striped peristome, contrast between the color of peristome and that of the pitcher body, and contrast between the color of the pitcher lid and pitcher body. This kind of patterning seems likely to play a role in signaling to visually-oriented animals that can distinguish between red and green, and a contrasting pattern has already been shown to be important in signaling to vertebrate visitors in coprophagous species (*N. lowii*, *N. rajah*, and *N. macrophylla*: Moran et al., 2012). More generally, pitcher contrast may be important to anthophilous insects as well (*N. rafflesiana*; Moran, Booth, & Charles, 1999). We found more origins of peristome and lid contrast in upper pitchers than in lower pitchers (Figure 4), possibly because upper pitchers tend to be in higher light environments that could make such patterns more effective in signaling.

Correlations between altitude, habit, and habitat and morphological evolution

The potential ecological drivers we explored in our comparative analysis include altitude, growth habit, and habitat. None of the quantitative traits we examined significantly correlate to altitude in our phylogeny (Table 3). However, the trend of increasing lower and upper peristome rib heights in relation to maximum altitude is compelling given the wetness-dependent function of the peristome (Bohn & Federle, 2004; Bauer & Federle, 2009), which is favored in climates with greater precipitation (Moran et al., 2013; Schwallier et al., 2016). As precipitation increases with altitude, the trend of peristome rib height increasing with altitude also makes sense. Altitude, growth habit, and habitat are all proxies for multiple abiotic factors, so our inability to find significant results for most our morphological traits using these environmental traits could mean that abiotic factors are generally less important to pitcher evolution than biotic factors, or that our metrics do not accurately capture enough relevant environmental variables.

Conclusions:

Although much remains to be learned about functional diversity of *Nepenthes* pitchers in relation to diet and prey capture, even less emphasis has been placed on the adaptations used by the plant to deter its own enemies. Our analysis of *Nepenthes* pitcher coloration indicates that herbivory may play a role in maintaining pitcher color polymorphism, and should be explored further experimentally. Herbivory is an understudied subject in *Nepenthes*, with few publications directly addressing herbivores that attack *Nepenthes* (Clarke, 1997b; Merbach et al., 2007; Bauer, Rembold, & Grafe 2016). More generally, the role of anthocyanins as an herbivore defense remains unsettled (Menzies et al., 2016), so an improved understanding of the influence

of herbivores on pitchers' complex pigmentation strategies may yield novel insights into the broader use of red coloration in leaves—and such questions require polymorphic species as models (Gould et al., 2000; Menzies et al., 2016). The unique nutritional challenges of carnivorous plants in general (Givnish et al., 1984) adds weight to the importance of herbivore defense in their ecology; and the biphasic life history of climbing *Nepenthes* emphasizes how environmental context interacts with the potential defensive role of anthocyanins, as evidenced by the prevalence of redder lowers. It is our hope that this study will serve to both review the current state of knowledge of *Nepenthes* diversity and stimulate future phylogenetic explorations of this unique plant group.

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Figure 1. (A-D) Photographs showing polymorphism in *Nepenthes gracilis*: (A) green lower pitcher, (B) red lower pitcher, (C) green upper pitcher, and (D) red upper pitcher. An (E) upper and (F) lower pitcher of *N. rafflesiana* showing a second example of pitcher dimorphism. Note the difference in tendrils (black arrows) between morphs, which twine in uppers and grow gravitropically in lowers, and note the wings (white arrows) in lower pitchers, which are lacking or reduced in uppers. (G) A lower pitcher of *N. hamata*, indicating the peristome with tall ribs (blue arrow) (H) Diagram of a generalized *Nepenthes* plant with key morphological features labelled. Diagram of plants with (I,J) similar coloration between morphs, (K) redder lower pitchers, and (L) redder upper pitchers. Photo credits: A and C-G, K. Gilbert; B, S. Johnson-Freyd. Illustration credit: H-L, Abraham Cone.

Figure 2. A linear regression between canopy coverage and percentage of red pitchers for our January 2014 field observations in Kent Ridge Park, Singapore shows a negative correlation between red pigmentation and light environment. Each point represents a single patch. p=0.003.

Figure 3. Phylogeny displaying topology for the *trnK+PTR1* tree mapped with quantitative morphological traits from McPherson et al. (2009) and Bauer et al. (2012). Each taxon is placed into an altitude class based on its recorded maximum occurring altitude (McPherson et al. 2009): low (0-1000 m asl), medium (1001-2000 m asl), and high (2001-3520 m asl). The size of the circle icon corresponds to the relative magnitude of that trait for the given taxon. Line weights in the phylogeny are proportional to the posterior probability values of its subtending node.

Figure 4. Phylogeny displaying topology for the *trnK+PTR1* tree mapped with qualitative morphological traits from McPherson et al. (2009) data. Each taxon is placed into an altitude class based on its recorded maximum occurring altitude (McPherson et al. 2009): low (0-1000 m asl), medium (1001-2000 m asl), and high (2001-3520 m asl). Where information is available, the presence of the trait is represented by a black square and the absence of the trait with an open square. Color polymorphism is the exception as the sole non-binary qualitative trait. Here the three states are similar coloration between morphs (open square), redder lower pitchers (black square), and redder upper pitchers (grey square). Line weights in the phylogeny are proportional to the posterior probability values of its subtending node.

Figure 5. Evolutionary pathways of color polymorphism. Illustrated are the three states of color polymorphism we scored (from top, counterclockwise): similar coloration between pitcher morphs, lower pitchers more red-pigmented, and upper pitchers more red-pigmented. Arrows show direction of state change. Numbers above arrows represent the frequency of that transition in our character-mapped phylogeny. Note that the majority of transitions initiate from the "Redder Lowers" state. Illustration credit: Abraham Cone.

Table 1. Phylogenetic signal in continuous (quantitative) traits using Pagel's lambda (Pagel 1999) and Blomberg's K statistic (Blomberg et al. 2013), p-values are in parentheses. Lamina length, upper/lower pitcher length, and upper/lower peristome rib heights are values from McPherson et al. (2009). Peristome width (peristome width values corrected for pitcher length) and peristome slope (the length of the inward sloping portion of the peristome) are values taken from Bauer et al. (2012) for comparison. Values significant at the Bonferroni-corrected alpha value of 0.00625 are indicated by an asterisk. Significant values indicate a trait with phylogenetic signal.

Trait	lambda	K
Lamina Length	0 (p = 1)	0.11 (p = 0.4)
Lower Pitcher Length	0.22 (p = 0.31)	0.16 (p = 0.02)
Upper Pitcher Length	0 (p = 1)	0.09 (p = 0.48)
Lower Peristome Rib Height	0 (p = 1)	0.13 (p = 0.38)
Upper Peristome Rib Height	0 (p = 1)	0.13 (p = 0.33)
Peristome Width	0 (p = 1)	0.18 (p = 0.02)
Peristome Slope	0 (p = 1)	0.06 (p = 0.81)

Table 2. Phylogenetic signal in binary traits using Fritz and Purvis's D statistic. Given is the estimated D statistic for each trait (Fritz and Purvis 2010) as well as the probability that the trait is randomly distributed in the phylogeny (for a true random distribution D not significantly different from 1), and the probability that the trait is distributed according to a Brownian pattern (D not significantly different from 0). The extreme values for the D statistic are -2.4 for clumped and 1.9 for overdispersed. The scoring of these binary traits derived from McPherson et al. (2009) is described in the methods. "Similar Coloration", "Redder Lowers", and "Redder Uppers" are all elements of color polymorphism converted to binary. Values significant at the Bonferroni-corrected alpha value of 0.0035 are indicated by an asterisk.

Trait	Estimated D	prob_random	prob_brownian

Pitcher Dimorphism	1.13	0.567	0.056
Reduced Lower Pitchers	0.22	0.007	0.333
Similar Coloration	1.14	0.674	0.003*
Redder Lowers	0.93	0.401	0.008
Redder Uppers	0.53	0.251	0.383
Lower Lid Contrast	0.57	0.144	0.165
Lower Peristome Contrast	0.68	0.137	0.046
Lower Peristome Stripes	0.13	0.003*	0.393
Upper Lid Contrast	0.20	0.005	0.32
Upper Peristome Contrast	0.46	0.041	0.126
Upper Peristome Stripes	0.53	0.042	0.077
Lamina Indumentum	0.78	0.199	0.009
Pitcher Indumentum	0.85	0.277	0.009
Eyespots	0.74	0.243	0.106

Table 3: Correlations among quantitative traits using phylogenetic generalized least squares. Pearson's correlation coefficients are in bold, p-values are in parentheses. An asterisk indicates statistical significance at a Bonferroni-corrected alpha level of 0.0017.

	Min Altitude	Max Altitude	Altitudinal Range	Lamina Length	Lower Pitcher Length	Upper Pitcher Length	Lower Peristome Rib Height
Min Altitude	**1**	-	-	-	-	-	-
Max Altitude	**0.53** (2.04E-7*)	**1**	-	-	-	-	-
Altitudinal Range	**-0.47** (8.40E-6*)	**0.41** (1.00E-4*)	**1**	-	-	-	-
Lamina Length	**-0.25** (0.0224)	**-0.25** (0.0245)	**0.04** (0.7133)	**1**	-	-	-
Lower Pitcher Length	**-0.12** (0.2860)	**-0.19** (0.0884)	**-0.08** (0.4938)	**0.37** (5.27E-4*)	**1**	-	-
Upper Pitcher Length	**-0.03** (0.7656)	**-0.28** (0.0109)	**-0.30** (0.0062)	**0.19** (0.0789)	**0.27** (0.0152)	**1**	-
Lower Peristome Rib Height	**0.29** (0.0081)	**0.33** (0.0028)	**0.04** (0.7544)	**-0.13** (0.2325)	**0.26** (0.0207)	**0.03** (0.8004)	**1**
Upper Peristome Rib Height	**0.26** (0.0204)	**0.30** (0.0070)	**0.07** (0.5102)	**-0.11** (0.3344)	**0.12** (0.2532)	**0.08** (0.4632)	**0.95** (0.0000*)

Supplemental Table 1. List of taxa with Genbank accessions used in phylogenetic reconstructions. *This sample has been renamed as "*N. chaniana 3*" in this paper.

Taxon	*trnK*	*PTR1*	*ITS*
Ancistrocladus abbreviatus	AF315939.1		
Nepenthes adnata	AF315866.1	DQ840220.1	AB675864.1
Nepenthes alata	AF315891.1		AB675865.1
Nepenthes albomarginata	AF315908.1	DQ840224.1	
Nepenthes ampullaria	AF315888.1		AB675914.1
Nepenthes anamensis		DQ840225.1	
Nepenthes aristolochioides	DQ007088.1		
Nepenthes bellii	AF315926.1		AB675868.1
Nepenthes bicalcarata	DQ007089.1		AB675715.1
Nepenthes bongso	AF315865.1		AB675703.1
Nepenthes boschiana	AF315903.1	DQ840226.1	
Nepenthes burbidgeae	AF315921.1	DQ840227.1	AB675869.1
Nepenthes burkei	DQ840247.1	DQ840216.1	AB675870.1
Nepenthes cf petiolata HM2001	AF315902.1		
Nepenthes chaniana	KP152384.1		AB675872.1
Nepenthes chaniana 2	KP152385.1		
Nepenthes clipeata	AF315878.1	DQ840212.1	AB675873.1
Nepenthes danseri	DQ007087.1		AB675915.1
Nepenthes densiflora	AF315927.1	DQ840234.1	AB675875.1
Nepenthes diatas	AF315915.1	DQ840235.1	AB675876.1
Nepenthes distillatoria	AF315886.1	DQ840204.1	AB675877.1
Nepenthes dubia	AF315869.1		AB675698.1
Nepenthes edwardsiana	DQ840248.1	DQ840236.1	
Nepenthes ephippiata	AF315906.1	DQ840237.1	AB675878.1
Nepenthes eustachya	AF315867.1	DQ840238.1	AB675702.1
Nepenthes eymae	AF315930.1		AB675696.1
Nepenthes faizaliana	AF315917.1	DQ840239.1	AB675879.1
Nepenthes fusca	AF315936.1	DQ840240.1	AB675880.1
Nepenthes glabrata	AF315928.1	DQ840222.1	AB675881.1
Nepenthes gracilis	AF315937.1	DQ840241.1	AB675882.1
Nepenthes gracillima	DQ007086.1		
Nepenthes gymnamphora	AF315864.1	DQ840214.1	AB675694.1
Nepenthes hamata	AF315914.1	DQ840221.1	
Nepenthes hirsuta	AF315889.1	DQ840242.1	AB675916.1
Nepenthes inermis	AF315870.1	DQ840243.1	AB675701.1
Nepenthes insignis	AF315881.1	DQ840210.1	
Nepenthes insignis 2	AF315882.1		
Nepenthes khasiana	AF315887.1	DQ840208.1	AB675883.1
Nepenthes lamii	AF315905.1		
Nepenthes lavicola	AF315935.1	DQ840219.1	

Nepenthes longifolia	AF315871.1		AB675885.1
Nepenthes lowii	AF315875.1		AB675695.1
Nepenthes macfarlanei	AF315894.1		
Nepenthes macrophylla	AF315931.1		
Nepenthes macrovulgaris	AF315934.1		
Nepenthes madagascariensis	AF315883.1	DQ840207.1	AB769064.1
Nepenthes mapuluensis	AF315918.1	DQ840206.1	
Nepenthes masoalensis	AF315884.1		
Nepenthes maxima	AF315913.1	DQ840244.1	AB675697.1
Nepenthes merrilliana	AF315912.1		AB675887.1
Nepenthes mikei	AF315911.1		AB675700.1
Nepenthes mira	DQ007085.1		AB675711.1
Nepenthes mirabilis	AF315920.1		AB675889.1
Nepenthes muluensis	AF315933.1		
Nepenthes murudensis	DQ007084.1	DQ840223.1	
Nepenthes neoguineensis	AF315896.1		AB675917.1
Nepenthes northiana	AF315901.1		
Nepenthes ovata	AF315873.1		AB675892.1
Nepenthes pectinata	AF315909.1		AB675708.1
Nepenthes pervillei	AF315885.1		AB675893.1
*Nepenthes pilosa**	AF315919.1	DQ840209.1	
Nepenthes rafflesiana	AF315910.1		
Nepenthes rajah	AF315880.1		AB675895.1
Nepenthes ramispina	DQ007083.1		
Nepenthes reinwardtiana	AF315907.1		AB675896.1
Nepenthes rhombicaulis	AF315874.1		AB675897.1
Nepenthes sanguinea	AF315923.1		AB675898.1
Nepenthes sibuyanensis	DQ840246.1	DQ840218.1	
Nepenthes singalana	DQ007082.1	DQ840228.1	
Nepenthes sp HHM 2001 1	AF315938.1		
Nepenthes sp HHM 2001 2	AF315929.1	DQ840230.1	
Nepenthes sp HHM 2001 3	DQ840245.1		
Nepenthes spathulata	DQ007081.1	DQ840229.1	AB675900.1
Nepenthes spectabilis	AF315868.1		AB675901.1
Nepenthes stenophylla	AF315922.1	DQ840231.1	AB675903.1
Nepenthes sumatrana	AF315872.1	DQ840215.1	AB675904.1
Nepenthes talangensis	AF315924.1		AB675905.1
Nepenthes tentaculata	AF315932.1		AB675920.1
Nepenthes thorelii	AF315890.1	DQ840232.1	AB675712.1
Nepenthes tobaica	AF315899.1	DQ840233.1	AB675907.1
Nepenthes tomoriana	AF315898.1	DQ840205.1	AB675706.1

Nepenthes treubiana	AF315893.1		
Nepenthes truncata	AF315904.1		AB675908.1
Nepenthes veitchii	AF315895.1		AB675909.1
Nepenthes ventricosa	AF315892.1		AB675910.1
Nepenthes vieillardii	AF315897.1		
Nepenthes villosa	AF315925.1	DQ840211.1	AB675911.1
Nepenthes xiphioides	DQ007080.1	DQ840213.1	
Triphyophyllum peltatum	AF315940.1		

Supplemental Table 2. Summary of collections of pitcher infauna for Singapore summer 2014, numbers presented are means ± standard deviation.
[†]Four pitchers that became severely damaged are excluded from the second date's counts.

	First Collection Date			Second Collection Date				
Pitcher Type	**Culicidae**	**Other Larvae**	**Ants**	**Culicidae**	**Other Larvae**	**Ants**	**Mites**	**Flying Prey**
Lower Green	0.33 ± 0.50	0.11 ± 0.33	1.33 ± 1.80	0.50 ± 0.76	0 ± 0	6.13 ± 7.85	0 ± 0	0 ± 0
Lower Red[†]	2.93 ± 5.09	0.64 ± 1.25	12.36 ± 20.31	1.05 ± 1.86	0.27 ± 0.55	11.32 ± 17.82	1.73 ± 2.85	0.36 ± 0.73
Upper Green	2.10 ± 2.47	0.57 ± 1.19	6.17 ± 7.11	1.74 ± 3.19	0.29 ± 0.80	3.18 ± 6.20	0.18 ± 0.63	0.94 ± 1.58
Upper Red	2.80 ± 2.39	1.80 ± 2.95	22.00 ± 40.87	0.40 ± 0.55	0 ± 0	12.60 ± 15.60	0.40 ± 0.55	1.80 ± 1.79
All Pitchers	2.13 ± 3.60	0.59 ± 1.32	8.59 ± 16.95	1.21 ± 2.47	0.22 ± 0.63	6.42 ± 12.12	0.63 ± 1.76	0.67 ± 1.30

Supplemental Table 3. Results of Poisson regressions conducted on *N. gracilis* pitcher infauna counts. For each prey item type (ants, mites, and flying prey) and symbiont (culicids and other larvae), the counts were tested in a linear mixed model with all traits as fixed effects in one model and with plant nested with site as random effects. The numbers presented are the estimate for the fixed effect with p-values in parentheses.
*Significant at $p<0.05$

Trait	Ants	Culicid Larvae	Other Larvae	Mites	Flying Prey
Pitcher color	1.01 (0.35)	-0.28 (0.80)	1.61 (0.51)	1.59 (0.15)	1.21 (0.44)
Pitcher morph	0.75 (0.49)	-0.18 (0.88)	1.72 (0.47)	0.87 (0.37)	2.67 (0.10)

Connectedness	-0.56 (0.06)	-0.16 (0.48)	-0.58 (0.11)	0.39 (0.25)	-0.20 (0.48)
Pitcher size	0.02 (0.01)*	0.01 (0.55)	-0.01 (0.82)	-0.01 (0.52)	-0.04 (0.07)
Distance from the ground	-0.01 (0.18)	0.01 (0.18)	0.03 (0.13)	-0.01 (0.10)	0.01 (0.15)

Supplemental Table 4. Contingency table for field survey of *N. gracilis* color polymorphism. $p=8*10^{-25}$

	Upper	Lower	Total
Red	9	189	198
Green	334	290	624
Total	343	479	822

Supplemental Table 5: Results of test of correlated evolution (binary PGLMM) between color polymorphism (converted to binary state, presence or absence of "similar coloration") and dimorphism, reduced lower pitchers, and the six color pattern-related traits: upper and lower lid contrast, upper and lower peristome contrast, and upper and lower peristome stripes. Provided for each trait is the s2 value and correlation estimate, with p-values in parentheses. The s2 value is a measure of the phylogenetic signal in the residuals; s2 values further from zero indicate more significant phylogenetic signal in the residual, p-values are in parentheses. Values that are significant at the Bonferroni-corrected alpha value of 0.00625 are indicated with an asterisk.

Dependent Trait	s2	Correlation Estimate
Pitcher Dimorphism	5.32E-14 (0.500)	-2.64 (0.016)
Reduced Lower Pitchers	1.45 (0.013)	1.54 (0.009)
Lower Lid Contrast	1.90 (0.164)	0.39 (0.573)
Lower Peristome Contrast	3.50 (0.007)	0.15 (0.818)
Lower Peristome Stripes	4.64 (1.33E-12*)	-0.53 (0.358)
Upper Lid Contrast	3.52 (1.09E-09*)	-0.35 (0.585)
Upper Peristome Contrast	4.34 (8.63E-04*)	0.22 (0.744)
Upper Peristome Stripes	4.85 (1.42E-15*)	-0.68 (0.231)

Supplemental Table 6: Results of test of correlated evolution (binary PGLMM) between reduced lower pitcher production trait and dimorphism, three binary color polymorphism traits (presence/absence of "similar coloration", "redder lowers", and "redder uppers"), and the six color pattern-related traits: upper and lower lid contrast, upper and lower peristome contrast, and upper and lower peristome stripes. Provided for each trait is the s2 value and correlation estimate, with p-values in parentheses. The s2 value is a measure of the phylogenetic signal in the residuals; s2 values further from zero indicate more significant phylogenetic signal in the residual, p-values are in parentheses. Values that are significant at the Bonferroni-corrected alpha value of 0.00625 are indicated with an asterisk.

Dependent Trait	s2	Correlation Estimate
Pitcher Dimorphism	2.63E-14 (2.32E-06*)	3.42 (0.074)
Similar Coloration	1.45E-13 (0.500)	1.70 (0.003*)
Redder Lowers	1.83E-09 (0.500)	-1.72 (0.003*)

Redder Uppers	4.11 (0.056)	0.15 (0.909)
Lower Lid Contrast	2.78 (0.132)	-0.10(0.908)
Lower Peristome Contrast	3.53 (0.005*)	0.37 (0.623)
Lower Peristome Stripes	3.81 (3.50E-10*)	0.04 (0.946)
Upper Lid Contrast	4.51 (2.78E-12*)	0.73 (0.322)
Upper Peristome Contrast	5.14 (1.37E-04*)	1.25 (0.104)
Upper Peristome Stripes	4.89 (3.21E-15*)	-0.78 (0.258)

Supplemental Tables 7: Select results of tests of correlated evolution (binary PGLMM) against habit (terrestrial/epiphyte/lithophyte) and habitat (dipterocarp forest, peat swamp, heath forest, montane forest, scrub, cliff, mangrove, seasonal grassland, and degraded). Provided for each response trait is the s2 value, p-value of the s2 value, correlation estimate, and p-vale for the correlation estimate. The s2 value is a measure of the phylogenetic signal in the residuals; s2 values further from zero indicate more significant phylogenetic signal in the residual, p-values are in parentheses. P-values that are significant at the Bonferroni-corrected alpha value of 0.00417 are indicated with an asterisk.

Pitcher Dimorphism

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	3.53E-09	8.95E-18*	-0.17	0.876
Epiphyte	2.65	1.57E-09*	23.60	0.999
Lithophyte	1.79E-07	0.002*	15.04	0.993
Dipterocarp Forest	3.11	3.89E-05*	1.36	0.235
Peat Swamp	9.08	0.001*	0.01	0.995
Heath Forest	0.20	0.361	0.95	0.385
Montane Forest	2.68	3.86E-06*	-0.89	0.431
Scrub	3.49	5.64E-09*	0.44	0.573
Cliff	1.08	1.71E-04*	-0.54	0.462
Mangrove	0.19	3.72E-15*	1.57	0.363
Seasonal Grassland	0.09	0.002*	13.62	0.990
Degraded	1.67	0.028*	1.61	0.147

Reduced Lowers

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	1.23E-12	1.10E-05*	14.09	0.991
Epiphyte	0.28	1.04E-05*	5.90	0.002*
Lithophyte	1.92E-12	1.10E-05*	-18.22	0.997
Dipterocarp Forest	3.01	4.26E-05*	-1.07	0.158
Peat Swamp	5.75	3.23E-09*	-2.77	0.155
Heath Forest	0.64	0.187	-0.22	0.743
Montane Forest	1.91	3.19E-08*	4.33	0.024

Scrub	3.40	3.10E-08*	-0.10	0.876
Cliff	0.78	0.008	-1.94	0.016
Mangrove	0.28	5.82E-06*	-3.73	0.234
Seasonal Grassland	9.10E-13	1.10E-05*	-14.09	0.991
Degraded	1.23	0.055	-1.38	0.054

Color Polymorphism (Presence/absence of "Similar Coloration")

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	1.02E-12	3.62E-08*	15.33	0.993
Epiphyte	1.56	8.24E-05*	0.35	0.484
Lithophyte	8.84E-12	3.62E-08*	-16.05	0.992
Dipterocarp Forest	2.90	9.42E-05*	-0.41	0.479
Peat Swamp	9.38	0.001*	-0.83	0.419
Heath Forest	0.42	0.267	-0.06	0.916
Montane Forest	2.51	3.02E-05*	0.90	0.181
Scrub	3.41	8.67E-08*	-0.06	0.907
Cliff	1.28	4.53E-05*	0.16	0.755
Mangrove	0.27	3.57E-08*	-5.01	0.221
Seasonal Grassland	1.54E-12	3.62E-08*	-15.33	0.993
Degraded	1.50	0.055	-1.01	0.064

Lower Lid Contrast

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	3.14E-15	1.79E-19*	0.47	0.779
Epiphyte	1.89	7.24E-06*	0.32	0.672
Lithophyte	3.99E-07	0.497	1.34	0.292
Dipterocarp Forest	2.64	3.32E-04*	0.56	0.481
Peat Swamp	9.34	0.001*	-0.34	0.813
Heath Forest	0.74	0.181	-0.30	0.727
Montane Forest	2.88	4.40E-06*	-0.60	0.458
Scrub	3.56	2.00E-07*	2.19	0.061
Cliff	1.61	1.82E-06*	-1.37	0.141
Mangrove	1.78	4.71E-04*	-11.77	0.960
Seasonal Grassland	2.97E-14	1.79E-19*	-0.47	0.779
Degraded	2.15	0.003*	-0.25	0.748

Upper Lid Contrast

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	8.24E-11	6.54E-06*	14.13	0.991
Epiphyte	1.54	1.89E-04*	-0.46	0.453
Lithophyte	7.94E-09	0.495	1.13	0.431
Dipterocarp Forest	3.00	1.55E-05*	0.37	0.587
Peat Swamp	9.65	0.001*	-0.42	0.732

Heath Forest	0.74	0.257	-0.76	0.295
Montane Forest	1.97	0.003*	-1.42	0.032
Scrub	3.39	3.10E-07*	0.37	0.593
Cliff	1.47	1.11E-06*	-0.69	0.288
Mangrove	0.57	0.329	0.63	0.525
Seasonal Grassland	5.94E-16	6.54E-06*	-14.13	0.991
Degraded	1.84	0.006	-0.21	0.734

Upper Peristome Contrast

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	3.33E-13	3.17E-05*	14.08	0.991
Epiphyte	1.97	9.03E-07*	0.43	0.504
Lithophyte	2.67	2.66E-05*	-13.90	0.986
Dipterocarp Forest	3.15	9.01E-06*	1.35	0.057
Peat Swamp	8.57	0.002*	0.68	0.518
Heath Forest	0.51	0.193	0.44	0.492
Montane Forest	2.82	2.87E-06*	-0.54	0.449
Scrub	3.30	9.63E-08*	0.38	0.604
Cliff	0.94	0.001*	0.04	0.951
Mangrove	1.81	0.101	-0.44	0.727
Seasonal Grassland	5.39E-13	3.17E-05*	-14.08	0.991
Degraded	1.76E-09	0.500	1.12	0.046

Upper Peristome Stripes

Dependent Trait	s2	s2 p-value	Correlation Estimate	Correlation Estimate p-value
Terrestrial	1.81E-09	2.13E-12*	-16.80	0.994
Epiphyte	1.59	7.66E-05*	0.40	0.438
Lithophyte	17.56	5.63E-16*	6.56	0.221
Dipterocarp Forest	3.71	8.34E-05*	0.49	0.422
Peat Swamp	7.82	0.003*	-0.83	0.497
Heath Forest	0.05	0.484	1.04	0.056
Montane Forest	4.23	1.42E-09*	-0.82	0.235
Scrub	3.01	4.08E-06*	-0.69	0.221
Cliff	0.94	0.001*	0.00	0.996
Mangrove	0.54	0.328	-0.90	0.435
Seasonal Grassland	5.87E-14	2.16E-08*	-15.33	0.992
Degraded	0.66	0.165	-0.53	0.309